

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
2,2-DIBROMO-3-NITRILOPROPIONAMIDE

Chemical Code # 001749, Tolerance # 50141
SB 950 # 477

Original date: 10/10/2002
Revised: 8/25/03

I. DATA GAP STATUS

Chronic toxicity, rat:	Data gap, no study submitted
Subchronic, rat (oral)	No data gap, no adverse effect
Chronic toxicity, dog:	Data gap, no study submitted
Oncogenicity, rat:	Data gap, no study submitted
Oncogenicity, mouse:	Data gap, no study submitted
Reproduction, rat:	Data gap, no study submitted
Teratology, rat:	Data gap, no study submitted
Teratology, rabbit:	Data gap, study inadequate, possible adverse effect indicated
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.
All record numbers through 202570 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T030825

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

CHRONIC TOXICITY, RAT

No study submitted.

CHRONIC TOXICITY, DOG

No study submitted.

ONCOGENICITY, RAT

No study submitted.

ONCOGENICITY, MOUSE

No study submitted.

REPRODUCTION, RAT

No study submitted.

TERATOLOGY, RAT

No study submitted.

TERATOLOGY, RABBIT

50141-039; 135581; "Biobrom C-103: Teratology in The Rabbit", LSRI Project No. DSB / 089 / BBR; Rubin, Y. and Nyska, A.; Life Science Research Israel Ltd., Israel ; 4/17/90. Biobrom C-103, (batch no. RES-No 625, purity not stated) was given by intragastric gavage to at concentrations of 0, 2, 10, 30, or 60 mg/kg/day to 14 HY/CR New Zealand White mated female rabbits/group on gestation days 7 through 19. Treatment related maternal effects at 60 mg/kg/day included mortality (6/13) and decreased food consumption, body weight gain and feces production (maternal NOEL = 30 mg/kg/day). Treatment-related fetal effects included increased incidence of reduced ossification of sternbrae and xipisternum and the long bone epiphyses at 30 and 60 mg/kg/day (developmental NOEL = 10 mg/kg/day). A possible adverse effect was indicated by the maternal NOEL > developmental NOEL. The study is unacceptable but possibly upgradeable with submission of statement of purity of the test material, batch no. RES-NO 625 (J. Kishiyama and S. Morris, 3/21/02).

50141-031 116007. Duplicate of 135581.

GENE MUTATION

****50141-039; 135582**; "Biobrom C-103 (DBNPA), 2,2-Dibromo-3-Nitrilo-propionamide: Assessment of Mutagenic potential in Histidine Auxotrophs of *Salmonella typhimurium* (Ames Test)", DSB/135/BIO; Lehrer, S.; Life Science Research Israel Ltd., Isreal; 5/9/91. C-103 (sample no. 588, purity not stated) was tested in the Ames Assay at concentrations ranging from 0.125 to 25 ug/plate with and without metabolic activation (S9 fraction of liver homogenates from sodium phenobarbital / 3-methylcholanthrene induced male rats). The assay measured the frequency of prototrophic colonies arising from histidine auxotrophic strains of *Salmonella typhimurium* (TA100, TA98, TA1535, TA1537, TA1538) in 2 trials with 2 plates per treatment. Treatment-related increases in prototrophic colonies were not seen. No adverse effect was indicated. The study was acceptable (J Kishiyama and S. Morris, 3/21/02).

****50141-028; 092517**; "C-103: Assessment of Mutagenic Potential in Histidine Auxotrophs of *Salmonella typhimurium* (Ames Test)", LSRI Report No. DSB/062/C-103; Evenchik, Z.; Life Science Research Israel Ltd; 1/17/85. C-103 (batch no. 34089-37, purity not stated) was tested

in the Ames Assay at concentrations ranging from 0.016 to 10 ug/plate with and without metabolic activation (S9 fraction of liver homogenates from sodium phenobarbital / 3-methylcholanthrene induced male rats). The assay measured the frequency of prototrophic colonies arising from histidine auxotrophic strains of *Salmonella typhimurium* (TA100, TA98, TA1535, TA1537, TA1538) in 2 trials with 2 plates treatment. Treatment-related increases in prototrophic colonies were not seen. No adverse effect was indicated. The study was acceptable (J Kishiyama and S. Morris, 3/21/02).

****50141-015; 041882;** "Evaluation of 2,2-Dibromo-3-Nitrilopropionamide in The Chinese Hamster Ovary Cell/Hypoxanthine (Guanine) Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay", HET K-078141-022; Dow Chemical Co., Midland MI; 12/16/1985; A.L. Mendrala. 2,2-Dibromo-3-Nitrilopropionamide (DBNPA, 95% state purity, lot 12164-76) was tested by measuring the forward mutation rate of the hypoxanthine phosphoribosyl transferase (HGPRT) loci of Chinese hamster ovary (CHO) cells. CHO cells were plated at 2×10^4 cells/cm², incubated for 16-18 hours, washed with saline and incubated for 4-5 hours with the treatment solution with (+S9) or without (-S9) metabolic activation (S9 fraction of rat liver homogenates). Cultures were then washed with saline, incubated in complete media and subcultured at 16-24 hours and days 3 and 6. On day 8, cells were harvested, 5 plates/treatment were seeded at 2×10^5 cells/plate, and incubated in the presence of 10 uM 6-thioguanine (TG) for 7 days. Mutation rates were measured as the frequency of TG resistant colonies. Tests were conducted without S9 at 10, 12.5, 15, 17.5, 20, 25, 50, 75, 100, 150, 200, and 400 uM DBNPA and with S9 at 25, 50, 75, 100, 150, 200 and 400 uM. Positive controls were adequate. A treatment-related effect on HG resistant colonies was not observed. No adverse effect was indicated. The study was acceptable (J. Kishiyama and S. Morris, 3/23/02).

50141-014; 036856; "The Mutagenicity of DBNPA Dissolved in Polyethylene Glycol in the Ames Test", GHE-T-063-K-78141; de Raat, W.K.; TNO-DELFT, The Netherlands; 7/2/1982. Slimicide XD 7287 (20% 2,2-dibromo-3-nitrilopropionamide [DBNPA] in polyethylene glycol:water vehicle) was tested in the Ames Assay at concentrations ranging from 0.005 to 0.50 mg/plate with and without metabolic activation (S9 fraction of liver homogenates from Aroclor 1254-treated rats). The assay measured the frequency of prototrophic colonies arising from histidine auxotrophic strains of *Salmonella typhimurium* (TA100, TA98, TA1535, and TA1537) in three plates per dose. A possible adverse effect was indicated by increased numbers of prototrophic colonies of TA 98 at 0.5 mg/plate +S9, 0.1 mg/plate -S9 and TA 100 at 0.5 mg/plate +S9 and 0.04, 0.05 and 0.06 mg/plate -S9. The study is acceptable (J Kishiyama and S. Morris, 3/21/02).

**** 0068; 202569;** "Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with 2,2-Dibromo-3-Nitrilopropionamide (DBNPA)"; (M.S. Mecchi; Covance Laboratories, Vienna, VA; Study No. 22647-04220ECD; 5/7/02); *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA were preincubated for 20 ± 2 minutes followed by treatment for 52 ± 4 hours at 37°C with 2,2-Dibromo-3-Nitrilopropionamide (DBNPA) (lot no. OF0801QT01, purity: 99.4%) at concentrations ranging from 0.333 to 100 ug/plate with and 0.0333 to 33.3 ug/plate without S9 activation. All treatment levels of the test article, the vehicle controls and the positive controls were plated in triplicate. Aroclor 1254-induced rat liver S9 fraction was used to activate the test material. Positive controls were functional. The test material did not produce a positive increase in the number of revertants per plate of any of the test strains either in the presence or absence of microsomal activation. The positive controls were functional. **No adverse effects. Study acceptable** (Moore, 4/4/03).

CHROMOSOME EFFECTS

****50141-039; 135583;** "Evaluation of the Ability of 2,2-Dibromo-3-Nitrilo-propionamide to Induce Chromosome Aberrations in Cultured Peripheral Human Lymphocytes", RCC NOTOX 008459; Enninga, I.C.; Research and Consulting Company B.V. NOTOX, Netherlands; Feb., 1989. Samples of proliferating cultured human lymphocytes were exposed to C-103 (2,2-Dibromo-3-

Nitrilopropionamide, DBNPG, lot 23/1817, 98% stated purity) with and with metabolic activation (S-9 fraction of Aroclor-1254 induced rat liver homogenates) for 2 hours, incubated for 22 or 46 hours, arrested in metaphase with colchicine, fixed, stained and microscopically examined for chromosome aberrations. 100 cells were evaluated per sample with two samples per treatment. Treatment levels without S-9 were 0, 25, 37.5 and 50 µg/ml (22 hour incubation) and 0 and 37.5 µg/ml (46 hour incubation) and with S-9 were 0, 5, 25, and 50 µg/ml (22 hour incubation) and 0 and 37.5 µg/ml (46 hour incubation). Positive controls were adequate. No treatment related effects on chromosome aberrations were reported. No adverse effect was indicated. The study was acceptable (J Kishiyama and S. Morris, 3/21/02).

**50141-015; 041880; "Evaluation of 2,2-Dibromo-3-Nitrilopropionamide (DBNPA) in The Mouse Bone Marrow Micronucleus Test," TXT: K078141-020; R.J. Bruce, B.B. Collapudi, and J.E. Wilkerson; Dow Chemical Co., Freeport, TX; 10/10/85. Groups of 5 CD⁻¹ (ICR) BR mice/sex were given single oral gavages of DBNPA (lot # 12164-76, 95%, water vehicle) at 0, 9, 30, and 90 mg/kg. Five mice/group were sacrificed 24 and 48 hours after treatment, femur bone marrow samples were fixed on microscope slides and stained. One thousand polychromatic erythrocytes /mouse were microscopically evaluated for micronuclei. The frequency of micronuclei was not treatment-related. No adverse effect was indicated. The positive controls were adequate. The study was acceptable (J. Kishiyama and S. Morris, 2/7/2002).

DNA DAMAGE

**50141-039; 135584; "Unscheduled DNA Synthesis in Primary Hepatocytes of Male Rats *In vitro* with Biobrom C-103", CCR 145405; Fautz, R.; L M P, Laboratory for Mutagenicity Testing; 5/8/89. Samples of primary rat hepatocytes attached to plastic cover slips were exposed to Biobrom C-103 (98% stated purity, batch 23) at 0, 0.08, 0.23, 0.77, 2.30, and 7.67 µg/ml in the presence of ³HTdR for 18 hours. Nuclei were swelled, cells fixed and developed for micro autoradiographic analysis of net nuclear grain counts in 50 nuclei per cover slip. There were 2 trials with 2 cover slips per treatment. Cytotoxicity was seen at 7.67 µg/ml. Net nuclear grain counts were not treatment related. No adverse effect was reported. The study was acceptable (J Kishiyama and S. Morris, 3/21/02).

**50141-015; 041881; "The Evaluation of 2,2-Dibromo-3-Nitrilopropionamide in The Rat Hepatocyte Unscheduled DNA Synthesis Assay," HET K-078141-021; A.L. Mendrala; Dow Chemical Co., Midland, MI; 12/13/85. Primary rat liver hepatocytes were isolated by collagenase perfusion of male CDF Fisher 344 rat livers. Samples of hepatocytes were treated with DBNPA (95% stated purity, lot # 12164-76, DMSO solvent) at 0, 4x10⁻⁶, 1.26x10⁻⁵, 4x10⁻⁵, 1.26x10⁻⁴, 4x10⁻⁴, 1.26x10⁻³, and 4x10⁻³ M for 18 hours in the presence of ³H-thymidine. Cells were fixed and stained on cover slips and developed with photographic emulsion. Unscheduled DNA Synthesis (UDS) was quantified as the mean net nuclear grain counts on the microautoradiographs of 30 hepatocytes / dose. Cytotoxicity was observed at doses 1.26x10⁻⁵ M and greater. The positive controls were adequate. Treatment related increases in UDS were not observed. No adverse effect was indicated. The study was acceptable (J. Kishiyama and S. Morris, 3/21/02).

50141-014; 036855; "An Investigation into the Induction of Sister-Chromatid Exchange by Slimicide XD 7287", Report No. CL 81/163; Davis, P.B.; TNO-DELFT, The Netherlands; 11/23/81. Slimicide XD 7287 (20% 2,2-dibromo-3-nitrilopropionamide [DBNPA] in 55:45 polyethylene glycol:water vehicle) was evaluated for sister chromatid exchange (SCE) in Chinese hamster ovary cells after one hour exposures at DBNPA concentrations of 0, 0.5, 1.5, 5, 15, and 50 ug/ml with and without metabolic activation (S9 fraction of Aroclor 1254 induced male Small Wistar rat liver homogenates). Significant treatment-related effects on SCEs were not seen. No adverse effect was indicated. The study was unacceptable and not upgradeable because of inadequate replicates, inadequate rationale for the doses used, no raw data and no analytical data (J Kishiyama and S. Morris, 3/21/02).

NEUROTOXICITY

Not required at this time

SUPPLEMENTAL STUDIES

50141-048; 095243; "2,2-Dibromo-3-Nitrilopropionamide (DBNPA): 13-Week Dermal Toxicity Study in Fischer 344 Rats", Lab Project No. K-078141-028; M.J. Mizell, H.M. Firchau and R.J. Kociba; Dow Chemical Company, Toxicology Research Laboratory; October 5, 1990. A 20% solution of DBNPA (lot MM890624, tetraethylene glycol vehicle) was applied undiluted for 6 hours / day, 5 days / week for 13 weeks to a 5 X 5 cm clipped patch (occluded) on the backs of 10 Fischer 344 rats/sex/group at 0, 103, 309, or 1031 mg/kg/day (0.0, 0.4, 1.2 or 4.0 ml/kg/day). Adequate ophthalmology, hematology, clinical chemistry, and urinalysis were performed with no treatment-related findings. A modified FOB was also included. Treatment related responses were localized to the application site: transient dermal irritation at 309 mg/kg/day and dermal irritation, erythema, edema, scabs, hyperkeratosis and inflammation at 1031 mg/kg/day (dermal NOEL = 103 mg/kg/day, systemic NOEL \geq 1031 mg/kg/day). The study was unacceptable but upgradeable with justification for using 20% test material. No worksheet was done (S. Morris, 10/10/02).

****50141-038; 135580;** "Biobrom C-103: Repeated Dose Oral Toxicity in the Rat: A 13-Week Subchronic Study", LSRI Report No. DSB/087/BBR; Crown, S.; Life Science Research Israel Ltd., Israel; 10/86. 2,2-Dibromo-3-Nitrilopropionamide (DBNPA, Biobrom C-103, 100% stated purity, Res no. 625, water vehicle) was given by oral gavage at 0, 5, 13 or 35 mg/kg/day, 7 days/week for 13 weeks to 20 Sprague-Dawley CD rats/sex/group. The 35 mg/kg/day dose was reduced after 4 days of treatment to 30 mg/kg/day due to dyspnea in both sexes. Treatment related effects were dyspnea, increased urine volume in males at 30 and 13 mg/kg/day and increased mortality, dyspnea, plasma urea, creatinine, adrenal weight, urine volume, tympanism of the digestive tract, hemorrhage of the lungs, acute tracheitis and depressed lymphoreticular system at 35(30) mg/kg/day (NOEL = 5 mg/kg/day). There was no effect on ophthalmology or histopathology other than decedents. The study was acceptable. No worksheet was done (S. Morris, 10/10/2).

50141-014; 036854; "A 90-Day Toxicological Study of Rats Maintained on Water Containing 2,2-Dibromo-3-Nitrilopropionamide", NB T11.10-78141-6; Humsiton, C.G., C.E. Wade, R.J. Kociba, S.B. McCollister, and B.A. Schwetz; The Dow Chemical Company; 9/16/71. 2,2-Dibromo-3-Nitrilopropionamide (DBNPA, XD-1603L, 95.7% stated purity, run 09020-24) was given at 0, 20, 100, or 500 ppm in the drinking water (pH 4 or pH 8) for 90 days to 10 Sprague-Dawley Spartan rats/sex/group. DBNPA is unstable at pH 8 and exposure was to breakdown products. The only treatment related effect of toxicological concern was minimal renal tubular alterations of the kidneys (minimal cytoplasmic swelling and vacuolization) in female rats maintained at 500 ppm, at pH 8 (NOEL = 100, pH 8; male, 8 mg/kg/day; female 15.9 mg/kg/day). The study was unacceptable and not upgradeable because of inadequate hematology, clinical chemistry, ophthalmology, and histopathology data. No worksheet was done (S. Morris, 10/10/2).

50141-0069; 202570; "Dibromonitrilopropionamide: 90-Day Dietary Toxicity Study in Fischer 344 Rats"; (B.L. Yano, J. Thomas, and P.C. Baker; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Study ID. 011175; 11/27/02); Ten Fischer 344 rats/sex/group were treated in the diet with a nominal 0, 3, 10, 100, 300, 600 or 1000 mg/kg/day of 2,2-Dibromo-3-Nitrilopropionamide (DBNPA) (lot no. PE2801QT01, purity: 99.65%) for up to 90 days (treatment levels determined analytically: (M) 0, 1.22, 4.6, 45, 133, 254 and 388 mg/kg/day, (F) 0, 1.17, 4.3, 44, 130, 251, 392 mg/kg/day). The discrepancy between the nominal doses and the actual test material uptake was due to the poor stability of the test material in the dietary preparations despite the diets being prepared weekly. The males in the 1000 mg/kg/day

group were euthanized on day 38 and the males in the 600 mg/kg/day group and the females in the 1000 mg/kg/day group were euthanized on day 73. These groups were terminated prior to the end of the study due to minimal body weight gain and a concern that a large number of the animals would not survive to the conclusion of the study. The mean body weights of the 300 mg/kg males and the 600 mg/kg females were less than that of the controls at the termination of the study ($p < 0.05$). Food consumption for both of these groups was also lower than that of the controls ($p < 0.05$). In the hematology evaluation, the mean red blood cell count for the 300 mg/kg males was lower than that of the control ($p < 0.05$) and the mean hemoglobin concentration for this group and the 100 mg/kg males was increased ($p < 0.05$). No effects were noted for the females in the hematology evaluation. In the clinical chemistry, the serum chloride levels were increased for the 100 mg/kg males and females and above ($p < 0.05$). In the urinalysis, there was a dose-related increase in the urinary ketone content at 100 mg/kg and above for both sexes. In the necropsy examination, the mean absolute weights for the heart, kidney, liver, brain, testes and epididymides of the 300 mg/kg males were less than those of the controls ($p < 0.05$). However, only the relative liver weight was lower for this group ($p < 0.05$). The relative brain weight was increased for this group of males ($p < 0.05$) and the relative spleen weights for the 100 and 300 mg/kg males were increased over that of the controls ($p < 0.05$). For the 600 mg/kg females, the mean absolute weights for the adrenal gland, heart, kidney, brain, ovaries, thymus and uterus were lower than those of the controls ($p < 0.05$). However, only the mean relative weight for the adrenal glands, ovaries, thymus and uterus of this group were lower than those of the controls ($p < 0.05$). The mean absolute spleen weights for the 100 and 300 mg/kg females were greater than that of the controls ($p < 0.05$). The mean relative spleen weights for the 300 and 600 mg/kg females were greater than that of the controls ($p < 0.05$). In the histopathological examination, very slight vacuolization was noted in the cortex of the adrenal glands of the 100 and 300 mg/kg males (0: 0/10 vs. 100: 7/10, 300: 9/10). Very slight or slight diffuse hyperplasia of erythroid cells in the bone marrow was noted for the 300 mg/kg males and the 600 mg/kg females ((M) 0: 2/10 vs. 300: 9/10, (F) 0: 0/10 vs. 600: 10/10). Focal or multifocal axonal degeneration of the optic chiasma in the brain was noted for the 300 mg/kg males (0: 0/10 vs. 300: 2/10). In conjunction with this lesion, slight or moderate unilateral axonal degeneration of the optic nerve and moderate unilateral retinal atrophy in the eye were noted for the 300 mg/kg males (0: 0/10 vs. 300: 2/10). For the 600 mg/kg females, there was an increased incidence of very slight or slight multifocal unilateral axonal degeneration of the optic nerve (0: 1/10 vs. 600: 3/10). Very slight or slight diffuse erythrocytic extramedullary hematopoiesis in the spleen was noted for the 300 mg/kg males (0: 0/10 vs. 300: 10/10) and the 3, 10, 100, 300, and 600 mg/kg females (0: 0/10 vs. 3: 1/10, 10: 2/10, 100: 4/10, 300: 9/10 and 600: 10/10). Unilateral or bilateral multifocal degeneration of the seminiferous tubules was noted in the testes of the 300 mg/kg males (0: 0/10 vs. 300: 2/10). The 600 mg/kg females suffered slight atrophy of the cervix and ovaries (cervix: 0: 0/10 vs. 600: 9/10, ovaries: 0: 0/10 vs. 600: 10/10). Very slight atrophy of the thymal cortex was noted for the 600 mg/kg females (0: 0/10 vs. 600: 8/10). **Possible adverse effect:** incidence of axonal degeneration in the optic chiasma, optic nerve and retinal atrophy in the eye; **Reported Subchronic NOEL:** (M/F) (Nominal) 10 mg/kg (analytical) (M: 4.6 mg/kg/day, F: 4.3 mg/kg/day) (based upon the incidence of vacuolization in the adrenal gland of the 100 mg/kg males and the increased incidence of extramedullary hematopoiesis in the spleen and increased absolute weight of the spleen in the 100 mg/kg females); **Study unacceptable**, possibly upgradeable with the submission of data for the individual animals. (Moore, 4/3/03)